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Assessment of the Safety of Some On-The-Shelf Canned Food Products Using PCR-Based Molecular Technique

*Ogbulie, T.E.[1], Uzomah, A.[2] and Agbugba, M.N.[1]

ABSTRACT

Study on the assessment of bacteria load of some on-the-shelf canned food products bought from different shops in the open markets in Owerri, Imo State, Nigeria was undertaken. Eleven canned food products including 2 cans of tomato paste (TP), 2 of fish-in-tomato-sauce (FTS), 3 of sweet corn (SC), baked beans (BB), 1 of green peas (GP) and 2 kidney beans (KB) were collected randomly and used for the analysis. Culture-based identification depicts the presence of *Bacillus* sp., *Staphylococcus* sp., *Vibrio parahaemolyticus, Salmonella* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp. and *Escherichia coli*. Plasmid profiling of culture-dependent isolates showed visible bands except *Staphylococcus* sp., *Klebsiella* sp. and *Bacillus* sp. Randomly amplified polymorphism DNA [RAPD] profiling using varying oligonucleotide primers specific for identification of 16SrRNA genes of some organisms of public health concern, confirmed the presence of coliforms, *Staphylococcus* sp., *Vibrio* sp., *S. typhi* and S. *enteritidis* on gel electrophoresis field. In the context of possible risk of consuming contaminated canned food products, this study was designed to show that most on-the-shelf canned food products can possibly habour organisms of public health importance which can be ascertained through molecular characterization using PCR-based technique. There is also the possibility of these organisms posing food safety issues and pharmaceutical risks in case of possible out break, assayed through plasmid profiling of the culture-dependent isolates. A major concern in this study is the lack of adherence to food safety regulations. The products still been marketed on the shelves and analyzed in this study must have outlived the shelf stability period.

Keywords: RAPD, canned food, bacteria load, plasmid profiling, oligonucleotide primers.

Introduction

Food can be defined as any substance consumed to provide nutritional support for the body. (Springer 2002). As stated by Frazier and Westhoff (1994) humans need nutrients to stimulate growth, produce energy and maintain life. Food spoilage is a process in which food deteriorates to the point in which it is not edible to humans or its quality of edibility becomes reduced (Brock, 1999; Frazier and Westhoff, 1994). It therefore means that the original nutritional value, texture, flavour of the food are damaged in such a way that the food becomes harmful to people and unsuitable to eat.

Furthermore, spoilage may be due to one or more of the following: physical changes such as those caused by freezing, burning, drying, pressure and chemical reactions such as those not catalyzed by enzymes of the tissue or of microorganism and biological (Springer, 2002). However, if the microorganisms involved are pathogenic, their association with food supply is critical from a public health point of view.

[1] Department of Biotechnology, School of Science, Federal University of Technology, Owerri, Nigeria.

[2] Department of Food Science and Technology, School of Engineering and Engineering Technology, Federal University of Technology, Owerri, Nigeria.

* corresponding author: ogbulie_toochi@yahoo.com

Food may decay by putrefaction, fermentation and rancidity hence, should be preserved. Foods can be preserved in many ways such as canning, drying, freezing, vacuum treatment, sugaring, salt and pickling (Frazier and Westhoff, 1994). Other methods include, ionizing, radiation, high pressure, use of chemical preservatives and removal of microorganisms. Also, controlled growth of certain bacteria is also used to fight the decaying action of microorganisms. Canning and bottling help retain the original taste and flavour of the food and hence are commonly followed methods of food preservation (Desrosier, 2004).

Canned foods are sterilized before being placed on the grocery shelf but if the sterilization has been unsuccessful, contamination or food spoilage may occur (Desrosier, 2002). Swollen cans most times do occur and usually contain gas produced by members of the genus *Clostridium*.

Sour spoilage without gas is called flat-sour spoilage. *Lactobacilli* are responsible for acid spoilage where they break down the carbohydrates. The different kinds of food which can be preserved by canning include fruits, vegetables, milk, beans and legumes, meat and fish, soups and other prepared items. In the context of this write up, canning is the method of interest. During canning process, air is driven from the jar and a vacuum is formed as the jar cools and seals. According to the United States, Department of Agriculture (USDA), the only two methods of canning approved are water-bath canning and pressure canning. The idea of canning is to make food available and edible long after the processing time. The heating process during canning appears to make dietary fibre more soluble and therefore more readily fermented in the colon into gases and physiologically active by-products.

Canned tomatoes have higher available lycopene content (Springer, 2002).

To improve food safety for those who consume canned foods, government have enacted laws requiring alphanumeric codes being put on food cans during manufacture indicating information relevant to health, such as the date of canning. "Open Dating" (use of a calendar as opposed to a code) on a food product is a date stamped on a product package to help the store determine how long to display the product for sale. It can also help the purchaser to know the time limit to purchase or use the product at its best quality. Consequently, foods that have offensive odour, flavour or appearance should not be eaten. Apart from missing out on key nutrients, one is also prone to food borne diseases like botulism caused by the bacteria *Clostridium botulinum, Listeria* infection etc. (Dainty, 2004).

Nevertheless, many retailers in developing country like Nigeria sometimes do not meet up in selling off of some of their old stock which remains on the shelf to almost, and most often beyond the expiration. Some on the other hand do not adhere to the manufacturers' specifications in terms of storage and hence leave the canned food most times on the shelf under the sun during sale thereby exposing them to certain environmental conditions that can enable bacterial growth. This study was therefore designed to assess the bacteriological load of on-the-shelf canned food products with a view to ascertain the possible presence of organisms of health importance through molecular characterization using molecular-based technique and plasmid profiling of the isolated organism.

Materials and Methods

Procurement of samples

The test canned food samples were collected randomly from open shops in the open markets in Owerri Municipal, Imo State, Nigeria. They include 2 cans of tomato paste (TP), 3 of sweet corn (SC), 1 of baked beans (BB), 2 of kidney beans (KB), 2 of fish-in-tomato-sauce (FTS) and 1 of green peas (GP) products. The samples were taken to the laboratory using surface sterilized cellophane bags within one hours of collection.

Culture-based identification

Ten fold serial dilutions were aseptically carried out and an aliquot inoculated onto sterile solidified agar

in sterile Petri dishes using spread plate method of Cheesbrough (1994). Pure cultures were thereafter obtained by streaking, Grams reaction of the bacterial isolates determined, followed by biochemical tests for identification of probable isolates carried out as described in Berger's Manual of Determinative Bacteriology (Holt, 1984).

Chromosomal DNA extraction

Chromosomal DNA extraction was carried out using modified boiling method of Sambrook and Russell (2001). This was carried out by first preparing a broth culture of isolates through 10 folds serial dilution of the canned food samples and inoculation of an aliquot into Luria Bertani (LB) medium. Thereafter, 1.5 ml of broth culture was added into each of the 11 pre-labelled eppendorf tubes. The tubes were vortexed for 2 min at 3000 rpm and then centrifuged at 10,000 rpm for 5 min. The supernatant were discarded and blotted on a paper towel. The cells were washed twice with 1 ml sterile distilled water and blotted after each wash. Two hundred micro litres (200 µL) of TE (Tris EDTA) buffer was added and vortexed to homogenize the pellets. The tubes containing the chromosomal DNA were heated in a dry hot microfuge block (Techne Dri Block DB- 3A) for 10 min, vortexed again and centrifuged at 12000 rpm for 5 min. The supernatant were transferred into a corresponding pre-labelled eppendorf tubes and the cell debris discarded. The DNA concentrations and purity was checked using Nanodrop spectrophotometer (Nanodrop model Nd 1000) and stored at -20^{o}C.

Plasmid DNA extraction by TENS method

Plasmid DNA of the cultured isolates was extracted using TENS method of Kraft *et al* (1988) and Sambrook and Russell (2001). This was carried out using a pre-labelled eppendorf tubes into which 1.5 ml of broth culture harvested from nutrient agar sub-cultured plates was added. The broth cultures were mixed by vortexing. They were then centrifuged at 13,000 rpm for 2 min and the supernatant discarded leaving a little of the broth. The pellets were again vortexed until they were completely suspended in the broth. Three hundred micro litre (300 µl) of TENS solution (Tris 25mM, EDTA 10mM, NaOH 0.1N and SDS 0.5%) was added to each of the tubes and mixed by inverting the tubes until the solution becomes slimy. Into each of the tubes, 150 µl of sodium acetate pH 5.2 was added and vortexed for 10 sec. The solution was spinned at 13000 rpm for 5 min. The supernatant were transferred into a corresponding pre-labelled eppendorf tubes and the cell debris discarded. Nine hundred micro litres (900 µl) of ice cold absolute ethanol was added into each of the tubes, vortexed and centrifuged at 13,000 rpm for 10 min. The supernatant were discarded and the white pellet observed. One millilitre (1 ml) of 70% cold ethanol was then added and centrifuged at 13,000 rpm for 5 mins. The last two steps were repeated. The pellets were air dried and 40 µl of TE buffer added and kept at -20^{o}C.

Polymerase chain reaction (PCR)

The PCR was carried out in a 20 µl reaction mixture containing A 5X HOT FIREPol Blend master mix (ready to use) composed of FIREPol® DNA polymerase proof-reading enzyme, 5X reaction buffer, 7.5mM $MgCl_2$, 1mM dNTPs of each have 200 µM of dATP, dCTP, dGTP, dTTP. A combination of 4 µl of master mix, 0.2 µl each of forward and reverse primers and 2 µl of template DNA constituted 6.4 µl. Hence 13.6 µl of sterile distilled water was added to make it up to the recommended PCR reaction mix of 20 µl. The entire mixture was then vortexed, pulse centrifuged and loaded together with positive and negative control (dH_2O) into the thermal cycler (eppendorf vapour protect). The PCR reaction was carried out with an initial denaturation at 95^{o}C for 5 min, followed by 30 consecutive cycles at 95^{o}C for 30 secs, varying annealing temperatures (depending on the primer used) for 1 minute and then 72^{o}C holding for 1 minute. This was then followed by a final extension step at 72^{o}C for 10 min. At the end

of the 30 cycles, the samples were loaded on 1.5% agarose gel for gel electrophoresis.

Analysis of PCR product by gel electrophoresis

After the PCR cycle, DNA bands were separated using a 1.5% agarose gel electrophoresis containing 50 µl of ethidium bromide. Two micro litre (2 µl) of loading dye was mixed with each 15 µl of PCR product and carefully loaded into the well created by the combs with 5 µl of 100bp DNA ladder loaded into the last/first well as marker. After loading of the gel, the electrodes were connected to the power pack. The electrophoresis was run at 80 v for 1 hour 30 minutes after which the DNA bands were viewed using UV transilluminator system (CLINX).

Results and Discussion

As the effect of microorganisms on human health has always been reported, the present study was performed to give information on the safety of some canned food product kept on the shelf in markets and to discuss their role of isolated microbes in food poisoning. The result of the culture-based bacteriological analysis and Gram staining is as shown in Table 2. Biochemical characterization of the test isolates as shown in Table 3 depicts the presence of *Klebsiella* species, *Staphylococcus* species, *Escherichia coli*, *Salmonella* species, *Bacillus* sp., *Pseudomonas* sp., *Proteus* sp. and *Vibrio parahaemolyticus* based on their biochemical reactions. Previous studies (Saadia and Hassanein, 2010) on isolation of pathogenic bacteria in food samples (milk and some fast foods) also indicated that some gram negative bacteria and gram positive bacteria were isolated as discovered in this investigation. Our result is in agreement with the above studies and is supported by research made by Kay *et al.* (1994) whose findings revealed some pathogenic bacteria found in fast foods and traditional fast foods. Most investigators indicated that bacteria, fungi and yeasts may exert their pathogenic action either through infection of body, or as a source of toxic substances demonstrated in contaminated foods (Saadia and Hassanein, 2010). The most common infections causing food poisoning and other diseases of man are those associated with contamination of food products (Frazier and Westhoff, 1994).

Table 1: Primer sequence of targeted genes (16SrRNA)

	Sequence	Primer annealing Temp	Microbes for targeted genes
Sef167F	5’ AGG TTC AGG CAG CGG TTA CT	50^0C	*Salmonella enteritidis*
Sef478R	5’ GGG ACA TTT AGC GTT TCT TG		
LZ – F	5’ATGAAAGCTGGCTACAGGAAGGCC	55^0C	all *Coliform*
LZ – R	5’GGTTTATGCAGCAACGAGACGTCA		
SJ- F	5’ GCC AAA AGA GAC TAT TAT GA	58^0C	*Staphylococcus* genera
SJ- R	5’ ATT GYT TAC CYG TTT GTG TAC C		
Sam 3 F	5’ CGGTGTTGCCCAGGTTGGTAAT	50^0C	*Salmonella* genera
Sam 4 R	5’ ACTCTTGCTGGCGGTGCGACTT		
Rxt A-F	5’ CTG AAT ATG AGT GGG TGA CTT ACG	50^0C	*Vibro* genera
Rxt A- R	5’ GTG TAT TGT TCG ATA TCC GCT ACG		
Fim A F	5’AGC CAA CCA TTG CTA AAA TGG CGC A	50^0C	*S. typhi*
Fim A R	5’ CCT TTC TCC ATC GTC CTG AA		
Com 1 F	5’ CAG CAG CCG CGG TAA TAC	58^0C	Bacteria genera
Com 2 R	5’ CCG TCA ATT CCT TTG AGT TT		
Shae F	5’ GTT GAG GGA ACA GAT	50^0C	*S. haemolyticus*
Shae R	5’ CAG CTG TTT GAA TAT CTT		

Table 2: Culture-based identification and grams reaction

Culture-based identification	Grams reaction	Suspected isolates
Smooth pink colonies on MacConkey agar	Gram negative rods	*Escherichia coli*
Large mucoid colony on MacConkey agar	Gram negative rod	*Klebsiella* species
Round whitish colony with glistering surface on nutrient agar	Gram positive cocci in clusters	*Staphylococcus* species
Black pigmented non-lactose fermenting colonies on Salmonella-Shigella agar	Gram negative rod	*Salmonella* sp.
Blue-green growth on Tiosulphate – citrate Bilesalt sucrose agar	Gram –ve slightly curved rod	*Vibrio parahaemolyticus*
Non-lactose fermenting colonies with a distinctive odour	Gram –ve rods	*Proteus* sp.
Grey to white colonies with rough edges on Nutrient agar	Gram +ve rods	*Bacillus* sp.
Round to spreading colony with yellowish green colouration on MacConkey agar	Gram –ve motile rods	*Pseudomonas* sp.

Table 3: Biochemical characterization of probable bacterial isolates

Citrate	Oxidase	Catalase	Indole	MR	VP	Glucose	Sucrose	Lactose	Fructose	Maltose	Mannitol	Probable	Genus
+	+	+	-	+	-	NT	NT	NT	NT	NT	NT	A	*Staphylococcus* sp.
-	-	+	+	+	-	+	AG	AG	AG	NR	A	A	*Escherichia coli*
+	-	+	-	+	-	+	AG	AG	AG	AG	A	A	*Klebsiella* sp.
+	+	-	-	+	-	+	NR	A	NR	NR	AG	NR	*Pseudomonas* sp.
+	-	+	-	NR	NR	+	NR	AG	NR	NR	AG	NR	*Bacillus* sp.
-	+	-	+	NR	NR	+	-	AG	NR	NR	NR	AG	*Vibrio parahaemolyticus*
-	-	NT	-	NR	NR	+	-	A	NR	NR	NR	A	*Salmonella* sp.
-	-	NT	-	+	NR	+	-	A	-	NR	NR	NR	*Proteus* sp.

-ve = negative; +ve = positive; + = positive reaction; - = negative reaction, Nr = no reaction; AG = acid and gas production; A = acid production, sp = species, NT = not tested.

Furthermore, report made elsewhere (Uyttendaele *et al.,* 1999) showed that raw food, especially meat, poultry, sea food and their juices, can contain microorganisms of public health concern, such as *Salmonella, Campylobacter, Listeria* and *Escherichia coli,* which may be transferred onto other foods during food preparation and storage. Isolation of *E. coli* and *Salmonella* species as observed in this study is in agreement with the above studies.

In raw milk and some other canned foods, the presence of lactic acid producing bacteria, under suitable conditions ferments the lactose present in the food to lactic acid. The increasing acidity in turn prevents the growth of other organisms, or slows their growth significantly. During pasteurization however, these lactic acid bacteria are mostly destroyed (Christison and Ivany, 2006; Chen *et al.*, 2007) which exposes the food substance to possible spoilage by any surviving spore forming bacteria, hence the food becomes contaminated and can cause food borne infections in man when not properly handled. This however, supports the finding of our study.

Molecular characterization

Molecular identification of some organisms of public health concern was carried out to confirm their possible presence in the test food samples. The presence of bacteria using com1 and 2 forward and reverse primers for 16 sRNA target gene for all bacteria was detected and obvious positive result was obtained as shown by the amplified bands (Fig. 1). Detection of the presence of coliforms, *Salmonella* and *Staphylococcus* genera in the test samples was also carried out using universal primer for the detection of such genus (Fig. 2 – 4). Specific primers for *Salmonella typhi*, *Salmonella enteritidis*, *Staphylococcus aureus* and *Staphylococcus haemolyticus* were also used to confirm their presence in the test samples. The results however revealed the presence of coliforms in the food samples (Fig. 2) indicated by visible amplified bands depicting the presence of coilforms of unknown genera. Fig. 3 shows the bacteria species detected in each sample of the canned product. Each 'lane' showed the different can product (1 and 4 – TP; 2 and 9 – KB; 3 and 5 – FTS; 6, 10 and 11 – SC; 7 – BB; and 8 – GP). The presence of *Staphylococcus* species was observed in lane 1, 2, 4 and 8 (Fig. 3). DNA of food samples in lane 1, 2, 3, 7, 8, 10 and 11 positive for general *Salmonella* species was further tested for amplification using `Sef 167/ Sef 478 primer for *S. enteritidis*. Bands were observed for lane 1, 2, 3, 8, and 9 though the bands are not highly amplified.

The plasmid DNA profiling of probable isolates were extracted and profiled as shown in Fig. 8. The result therefore indicate that the isolates in lane 3 (FTS) *(Vibrio)*, lane 6 (*Proteus* sp.), lane 7 (*Salmonella* sp.) lane 9 (*E. coli*) and lane 10 (*Pseudomonas* sp.) have plasmids. Although their plasmids have larger molecular weight except for the organism in lane 3 that has molecular weight of 23130 bp.

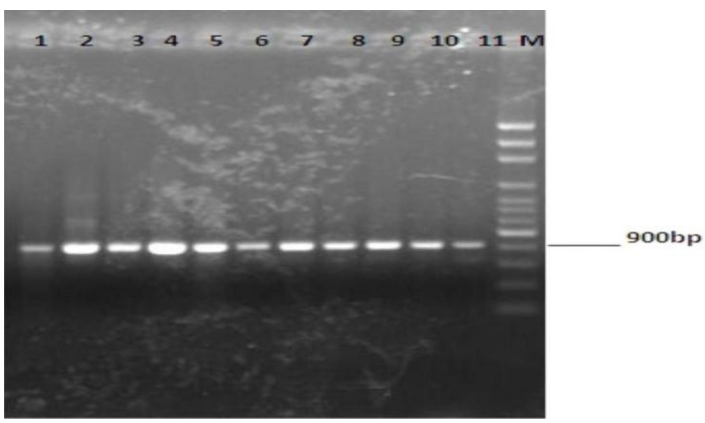

Fig. 1: Analysis of PCR product of the samples on gel electrophoresis showing amplified bands of isolates using DNA com 1 and 2 primer for all bacteria genera according to their molecular weights (legend for food samples: 1 & 4 (TP); 2&9 (KB); 3 & 5 (FTS); 6,10 &11- (SC); 7- (BB); 8- (GP))

Consequently, from this study, it is obvious that PCR-based technique can be used to detect the presence of microorganisms in canned foods samples as reported by Wiseman *et al.* (2009). The isolation of these organisms as reported in this study support the finding made by Narren *et al.* (2001), and could be as a result of under processing of the food and leaking of cans which are of major concern and both pose potential health risks. The isolation of *Escherichia coli, Staphylococcus species, Klebsiella species, Vibrio species, Pseudomonas species* in this study is in line with the findings of Landry *et*

al. (2001) who studied spoilage of canned foods. Their result showed the presence of the above mentioned microorganisms in addition to others in canned food. This study therefore shows that it is possible that some of the canned foods which are kept on the shelf in the market at varying storage temperature contain some microorganisms of health importance and which could be the reason why people complain of abdominal disorders eliciting gastrointestinal symptoms as abdominal cramp/pains, fever, diarrhoea, amoebic dysentery (*Proteus* sp.), nausea and vomiting; following the consumption of some of these canned food products. From this, it is note worthy that some of the observed organisms isolated from the canned food samples contain plasmids and this issue should not be taken with a grain of salt as the presence of the plasmids could be of a serious health issue and pharmaceutical risk in terms of antibiotic resistance.

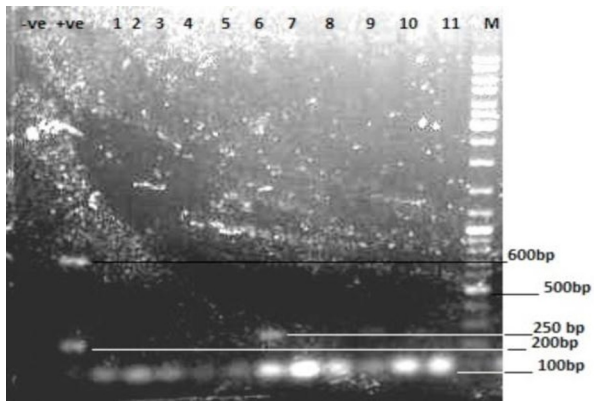

Fig. 2: Analysis of PCR product (using LZ primer for coliforms general) of the samples on gel electrophoresis showing amplified bands of isolates according to their molecular weights (legend for food samples: 1&4 (TP); 2&9 (KB); 3&5 (FTS); 6,10 &11- (SC); 7- (BB); 8- (GP))

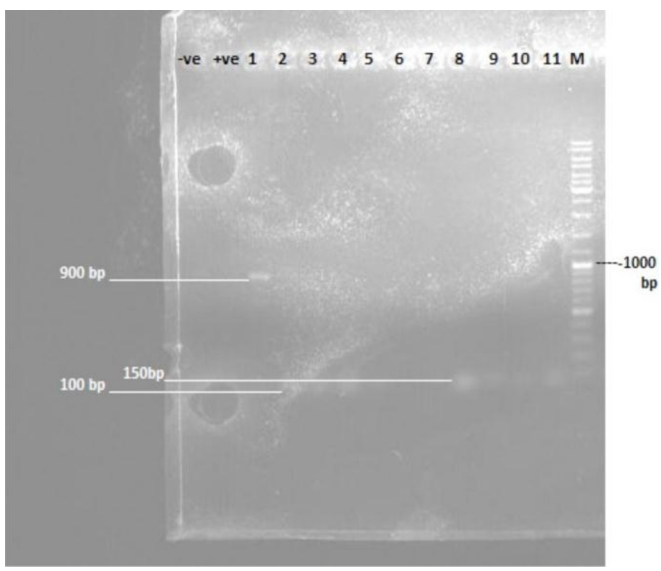

Fig. 3: Analysis of PCR product (using S-J primer for *Staphylococcus* species general) of the samples on gel electrophoresis showing amplified bands of isolates according to their molecular weights (legend for food samples: 1&4 (TP); 2&9 (KB); 3&5 (FTS); 6,10 &11- (SC); 7- (BB); 8- (GP))

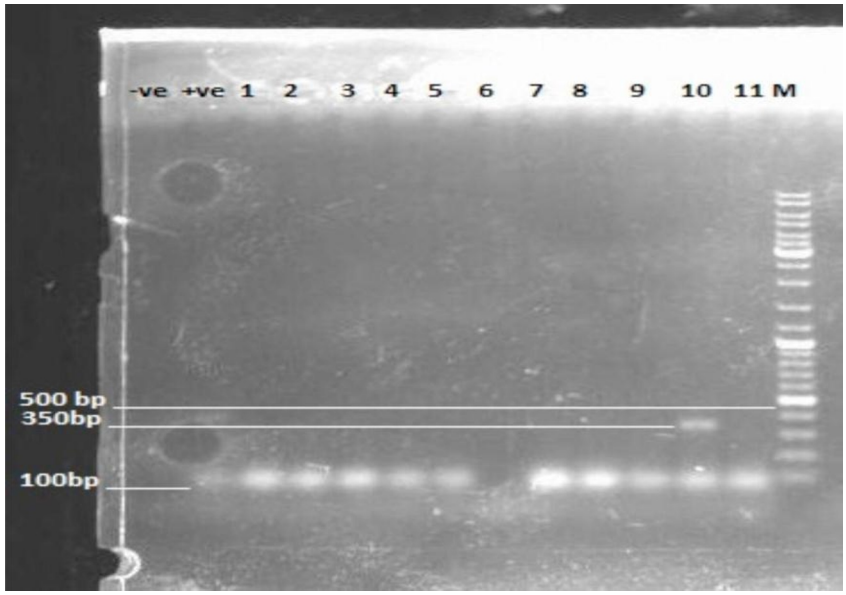

Fig. 4: Analysis of PCR product (using Sam 3 and Sam 4 primer for *Salmonella* species general) of the samples on gel electrophoresis showing amplified bands of isolates according to their molecular weights (legend for food samples: 1&4 (TP); 2&9 (KB); 3&5 (FTS); 6,10 &11- (SC); 7- (BB); 8- (GP))

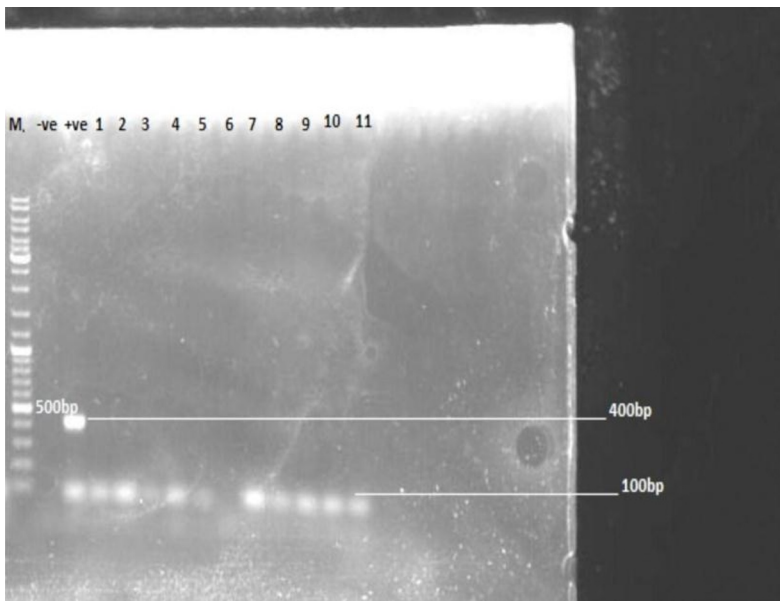

Fig. 5: Analysis of PCR product (using ST primer for *Salmonella typhii*) of the samples on gel electrophoresis showing amplified bands of isolates according to their molecular weights (legend for food samples: 1&4 (TP); 2&9 (KB); 3&5 (FTS); 6,10 &11- (SC); 7- (BB); 8- (GP))

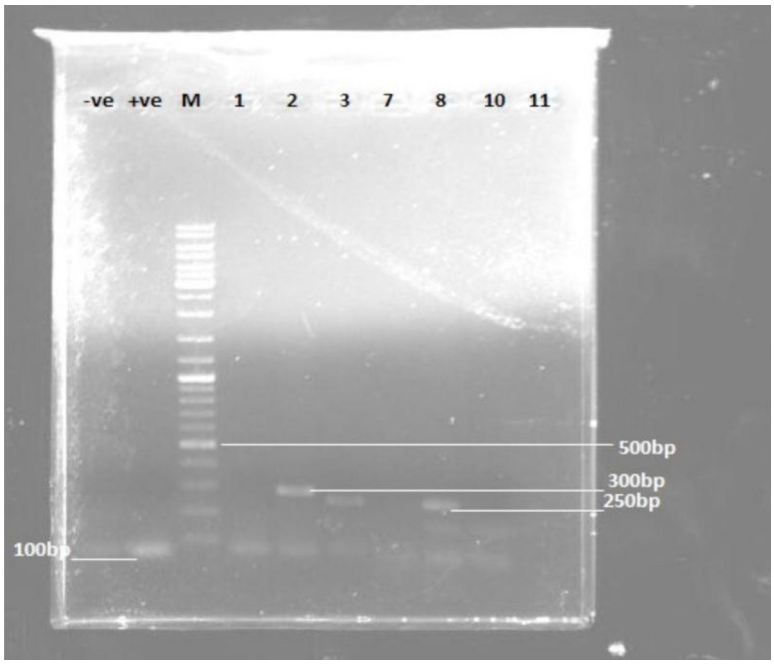

Fig. 6: Analysis of PCR product (using Sef 167/ Sef 478 primer for *Salmonella enteritidis*) of samples on gel electrophoresis showing amplified bands of isolates according to their molecular weights (legend for food samples: 1&4 (TP); 2&9 (KB); 3&5 (FTS); 6,10 &11- (SC); 7- (BB); 8- (GP))

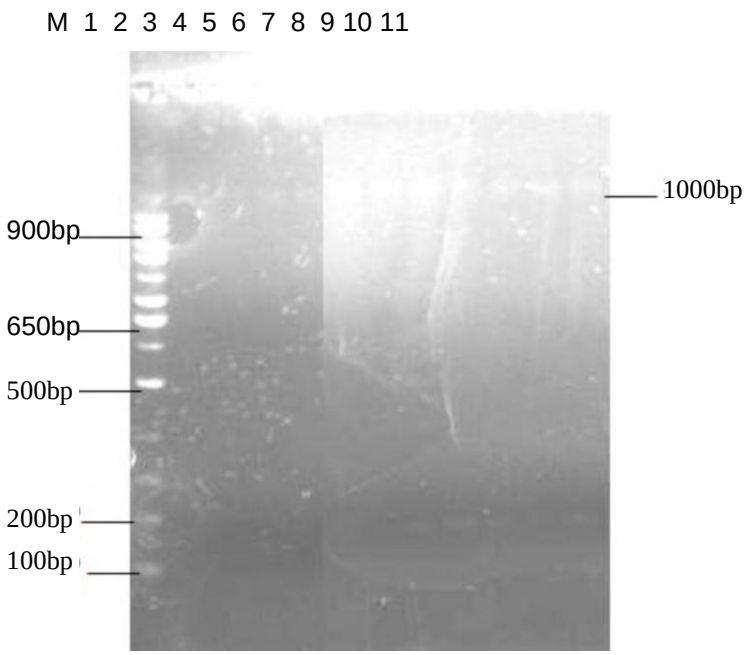

Fig. 7: Analysis of PCR product (using rxtA F and R primer for *Vibrio*) of samples on gel electrophoresis showing amplified bands of isolates according to their molecular weights (legend for food samples: 1 and 4 (TP); 2 and 9 (KB); 3 and 5 (FTS); 6, 10 and 11 (SC); 7- (BB); 8- (GP))

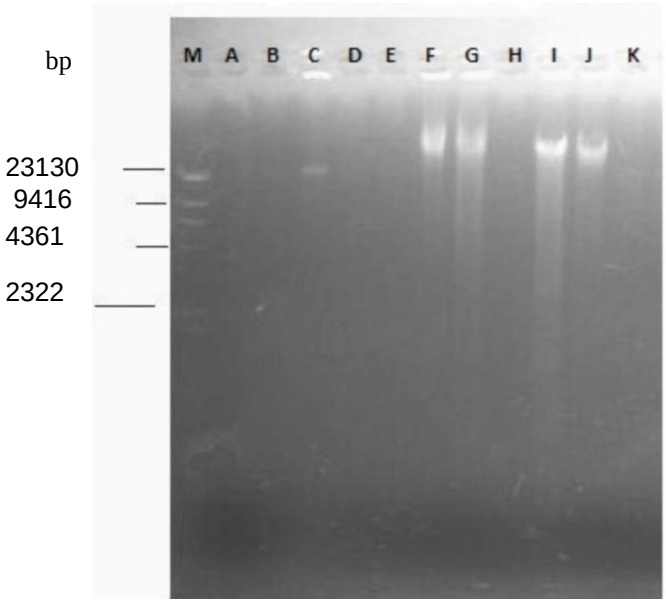

Fig. 8: Bands depicting plasmid profile of the cultured isolates from the canned food samples; A- *Bacillus* species, B- *Staphylococcus* species, C- *Vibrio*, D- *Staphylococcus* sp., E- *Staphylococcus* sp., F- *Proteus*, G- *Salmonella* species, H- *Klebsiella* sp., I- *E. coli*, J- *Pseudomonas*, K- *Klebsiella* and M - marker

Conclusion

To ensure safety of food products, quality control/ assurance through routine microbiological examination of food products for pathogens and spoilage organisms, confirmation of proper pasteurization and checking of leakages in cans are required. The expiry date on canned product is a good indication of the period the product can be regarded as good.

Subsequently, the risk of contamination under normal use conditions can be monitored and food poisoning out breaks can be prevented. PCR-based technique should be employed in carrying out some of the analysis in the laboratory of food agencies so that false conclusions are avoided. The need for good hygienic practices, proper handling, storage, display on shelves and retail of these canned foods cannot be overemphasized to ensure good quality and safe food product for consumers. The study however, promotes the need for proper sanitization and sterilization techniques to eliminate all the pathogenic microbes and reduce spoilage organisms to a level where they will not produce possible health risks or reduce the quality and acceptability of the food product.

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